



Elevated temperature and CO₂ affect responses of European aspen (*Populus tremula*) to soil pyrene contamination

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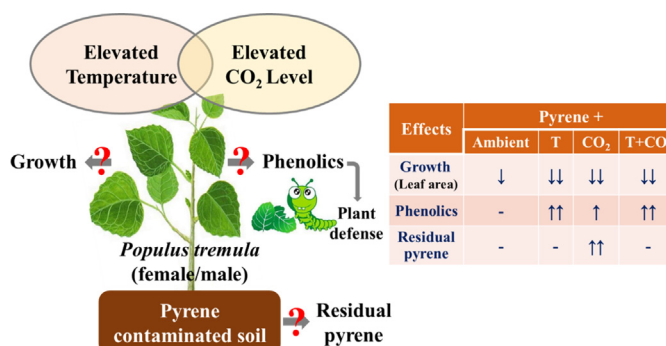
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HIGHLIGHTS

- Soil residual pyrene was higher under elevated CO₂ than under other treatments.
- Pyrene increased leaf phenolics of both sexes under elevated temperature and CO₂.
- Both sexes had better chemical defense capability to adjust to the combined stress.
- Potential risk of pyrene to plants will increase in the future changing climate.

GRAPHICAL ABSTRACT



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ABSTRACT

High northern latitudes are climatic sensitive areas, and are also regions to which polycyclic aromatic hydrocarbons (PAHs) easily transport and accumulate with potential risk to natural ecosystems. However, the effect of PAHs on northern woody plant growth and defense under climate change is very little studied. Here, we conducted a unique experiment in greenhouses to investigate sex-related responses of the dioecious *Populus tremula* to pyrene (50 mg kg⁻¹) and residue of pyrene in soils under ambient and elevated temperature (+1.8 °C on average) and CO₂ (740 ppm). Pyrene decreased stem biomass and leaf area by 9% and 6%, respectively under ambient conditions, and the reduction of leaf area was more severe under elevated temperature (38%), elevated CO₂ (37%), and combined T + CO₂ (42%). Other growth parameters were unchanged by pyrene. Pyrene did not affect the concentration of leaf total phenolics under ambient conditions, but increased it by 16%, 1%, and 20% compared to controls under elevated temperature, elevated CO₂, and T + CO₂, respectively. Pyrene had only minor sex-specific effects on plant growth and phenolics. The concentration of residual pyrene in pyrene-spiked soils was higher under elevated CO₂ than under ambient, elevated temperature, and combined T + CO₂. The results suggest that both sexes of *P. tremula* have the capacity to regulate growth and metabolism to adjust to the stress of the tested pyrene contamination under elevated temperature and CO₂, but potential risk of pyrene to plants still exists in the future changing climate.

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1. Introduction

The rapid economic development and increased human activities have increased anthropogenic greenhouse gas emissions. Especially, the atmospheric CO₂ concentrations have increased from 280 ppm in pre-industrial times to the current concentrations of 400 ppm, and will reach 430–1000 ppm by the year 2100 (IPCC, 2014; Way et al., 2015). Increasing emission of CO₂ and other greenhouse gases will lead to a rise in global average temperature of 1.5–4 °C by the end of this century relative to the level in 1850–1900, and the arctic region will continue to warm more rapidly than the global mean (IPCC, 2014). Temperature and CO₂ elevation have large impacts on plant performance. In general, slightly elevated temperature (e.g., +2 °C) enhances plant growth but decreases plant defensive phenolics under boreal conditions (Nybakken et al., 2012; Randriamanana et al., 2015). By contrast, elevated CO₂ increases plant growth and concentrations of phenolics under optimal conditions (Zhao et al., 2012; Lavola et al., 2013). Moreover, plant growth and phenolics are also affected by the interactions between elevated temperature and elevated CO₂, as one can modify the effect of the other (Veteli et al., 2007; Zhao et al., 2012; Nissinen et al., 2016). Elevated temperature and CO₂ may also affect the rhizosphere conditions, such as by decreasing the pH of rhizosphere soil, alter the composition or amount of root exudates, and change soil microbial communities (Rajkumar et al., 2013; Zhao et al., 2016).

Simultaneously with atmospheric temperature and CO₂ concentrations increase, soil contaminated with polycyclic aromatic hydrocarbons (PAHs) is becoming a global environmental problem. PAHs, a kind of persistent organic pollutants, are derived from natural and anthropogenic activities, especially the incomplete combustion of fossil fuels and biofuels (Desalme et al., 2013; Lamichhane et al., 2016). These emissions can be transported over long distances by atmospheric transport, and can enter soils by atmospheric deposition (Maliszewska-Kordybach et al., 2009; Friedman et al., 2014). PAHs easily accumulate in soils due to their hydrophobic properties, and they have been detected in woodland soils in the UK, Norway, Germany, and other European countries (Desaules et al., 2008; Nam et al., 2008; Holoubek et al., 2009; Aichner et al., 2015; Kuśmierz et al., 2016). Future climate change will affect concentrations of atmospheric PAHs. Friedman et al. (2014) simulated global atmospheric PAH concentration changes under future climate conditions using a chemical transport model GEOS-Chem. The results showed that phenanthrene and pyrene concentrations would increase slightly in the northern hemisphere in 2050. Thus, concentrations of soil PAHs may also be affected, and plants will inevitably be exposed to combined stress: soil PAHs contamination, elevated temperature, and elevated CO₂. PAH contamination can result in visible plant injuries (e.g., chlorosis, necrosis, and mesophyll collapse), decreased plant biomass, and perturbation of plant carbon allocation and photosynthetic efficiency (Desalme et al., 2013). However, the effects of PAHs on plants under elevated temperature and CO₂ are unknown. PAHs that enter soil may be lost or degraded by physico-chemical and biological processes, and the major means is biotic, i.e., through degradation or co-degradation processes mediated by bacteria or fungi (Kuppusamy et al., 2017). However, whether elevated temperature and CO₂ will affect the degradation of PAHs in soils is very little studied.

European aspen (*Populus tremula* L.) is widely distributed in Eurasian boreal and temperate ecosystems. It is a common dioecious species and the sex ratio is male biased in most of the distribution regions (Myking et al., 2011). Previous studies have reported sexual differences in plant growth, resource allocation, and responses to abiotic factors (Xu et al., 2008; Zhao et al., 2012; Randriamanana et al., 2014; Sobuj et al., 2018). Female plants usually invest more resources in chemical defense and reproduction, while male plants invest more in growth and are more susceptible to herbivore damage than females (Nybakken and Julkunen-Tiitto, 2013; Randriamanana et al., 2015; Maja et al., 2016). In addition, Randriamanana et al. (2015) noted that although males of

P. tremula were more growth-oriented under ambient conditions, the growth of females increased more under elevated temperature, and females had better chemical defense. Zhao et al. (2012) reported that elevated CO₂ enhanced plant growth in both males and females of *Populus cathayana*, but males benefited more from elevated CO₂. There may be trade-offs between growth and defense. For example, if elevated temperature and CO₂ increase only the growth and not the chemical defense in males, but increase both growth and chemical defense in females, males may be more susceptible to herbivore damage in the long run. However, we know little about the sex-specific responses to multi-factor stresses like PAHs contamination in combination with elevated temperature and CO₂.

In this study, our aim was to investigate the sex-related effects of soil PAH contamination on growth and defense of *P. tremula* seedlings under elevated temperature and CO₂. We chose a representative four-ring PAH with relatively higher potential genotoxicity and higher concentration (Tuhácková et al., 2001; Zhang and Chen, 2017), namely pyrene, to test the effects. The following questions were addressed. (1) Will soil pyrene contamination affect growth and defense of the dioecious *P. tremula*? (2) How are the effects of pyrene influenced by elevated temperature and CO₂? (3) Will the responses differ between females and males? (4) Will elevated temperature and CO₂ affect the residue of pyrene in soil?

2. Materials and methods

2.1. Experimental setup

The study was conducted at Mekrijärvi Research Station, Ilomantsi, University of Eastern Finland (62°47'N, 30°58'E, 145 m a.s.l.) from May 20 to August 5, 2015. Four greenhouses (16 m²) were assigned to four combinations of two-level temperature and CO₂ treatments: ambient temperature + ambient CO₂ (C), elevated temperature + ambient CO₂ (T), ambient temperature + elevated CO₂ (CO₂), and elevated temperature + elevated CO₂ (T + CO₂). Ambient temperature (15.2 °C on average) and CO₂ concentration (392 ppm on average) were achieved by following the outside air temperature and CO₂ concentration through a modulated system. Based on IPCC (2014), elevated temperature was set to 2 °C above the ambient level, and elevated CO₂ concentration was set to a doubling of the ambient level. The achieved temperature in the enhanced treatment greenhouses was on average 1.8 °C higher than that of the ambient level, and CO₂ concentration was on average 740 ppm. We also controlled the relative humidity at 60% in all four greenhouses. The photoperiod in the experimental greenhouses followed the outside natural day length. Detailed technical details are described in Zhou et al. (2012) and Nybakken and Julkunen-Tiitto (2013).

2.2. Soil preparation and plant materials

The peat soil (Kekkilä Oy, Finland) used in this experiment had no detectable pyrene, and the soil characteristics were as follows: pH, 6.2; available nitrogen (N), 0.7 g kg⁻¹; available phosphorus (P), 0.4 g kg⁻¹; available potassium (K), 1.5 g kg⁻¹; total Mg, 1.9 g kg⁻¹; conductivity, 18 ms s⁻¹. The soil was treated with pyrene (purity > 98, Sigma-Aldrich Co., USA) to have the concentration of 50 mg kg⁻¹. Pyrene in n-hexane was added to 10% (by volume) of the required quantity of soil. After n-hexane evaporated, the treated soils were progressively diluted with the remaining 90% (by volume) of untreated soils and mixed thoroughly. The pyrene-spiked soils were incubated for two weeks before planting. Soil without pyrene was used as the control soil.

The origins and the prophase of cultivation and acclimation of micropropagated *P. tremula* plantlets (eight genotypes, four females and four males) in this experiment were the same as in Sobuj et al. (2018). These seedlings were transferred to the greenhouses at

Mekrijärvi Research Station and planted in 4 L plastic pots on May 20, 2015. There were 256 plants (4 replicate seedlings \times 8 genotypes \times 2 soil treatments \times 4 greenhouses) in total for this study. All plants were watered regularly and fertilized twice (on June 4 and July 6) with Kekkilä Peat Superex (N: P: K = 12: 5: 27, %, Kekkilä Oy, Finland).

2.3. Growth measurements and sampling

We measured the height growth and diameter growth of all seedlings six times (1, 15, 29, 43, 57, and 71 days after planting). For leaf area measurements and phenolic analyses, two mature leaves from each seedling were collected at the end of the experiment and dried at room temperature in 10% relative humidity, in a drying room. Dried leaves were kept frozen (-20°C) until analyses. Leaf area (LA, cm^2) was measured by a portable leaf area meter LI-3000C (LI-COR, Lincoln, NE, USA), and specific leaf area (SLA, $\text{cm}^2 \text{g}^{-1}$) was calculated as the ratio of leaf area to its dry weight. For biomass determination, the aboveground parts of all seedlings were harvested on August 5, 2015, and dried at room temperature. They were then separated into leaves and stems and weighed. Following removal of plant tissues, the soil samples in each pot were collected, air-dried at room temperature and stored at -20°C until analysis.

2.4. Analyses of pyrene in soils and phenolics in leaves

Pyrene in soils was extracted with 1:1 (v/v) hexane/acetone and analyzed by high-performance liquid chromatography (HPLC, 1100 series, Agilent, USA) according to Gao et al. (2010). Pyrene was identified according to the retention time and UV spectrum of the standard, and quantified according to its absorbance at 234 nm. Phenolic compounds in leaves were extracted with methanol and analyzed by HPLC following the procedures described in Nybakken et al. (2012). Identification of leaf phenolics was performed by mass spectrometry using a quadrupole time-of-flight mass spectrometer (QTOF/MS, 6540 series, Agilent, USA) with an ultra-HPLC with a diode array detector (UHPLC-DAD, 1200 series, Agilent, USA) (Table S1). Detailed instrument conditions are described in Randriamanana et al. (2014). The reference mass m/z 922.0098 was used for accurate mass measurements. The mass accuracy or error term (ppm) was calculated as follows: (measured mass – calculated mass) $\times 10^6$ /calculated mass. Compounds whose mass could not be successfully determined were identified according to the retention times and UV spectra of corresponding standards, which were also used to calculate the concentrations: salicin (Sigma-Aldrich, Germany) for diglucoside of salicyl alcohol and salicin; chlorogenic acid (Sigma-Aldrich, Germany) for chlorogenic acid, neochlorogenic acid, and *p*-OH-cinnamoyl quinic acid; salicortin (Sigma-Aldrich, Germany) for salicortin and disalicortin; hyperoside (Roth, Germany) for quercetin 3-arabinoglucoside and quercetin 3-glucuronide; astragalol (Extrasynthese, France) for kaempferol 3-arabinoglucoside and kaempferol 3-glucuronide; tremulacin (Apin Chemicals, UK) for tremulacin; *p*-OH-cinnamic acid (Sigma-Aldrich, Germany) for *p*-OH-cinnamic acid derivatives, cinnamoyl salicortin, and *p*-OH-cinnamoyl salicortin; kaempferol 3-rhamnoside (Sigma-Aldrich, Germany) for monocoumaroyl-kaempferol 3-rhamnoside. Flavonoids and phenolic acids were quantified according to their absorbance at 320 nm and salicylates at 270 nm.

2.5. Statistical analyses

All data were analyzed by a linear mixed model with temperature, CO_2 , pyrene, and sex as fixed factors, and genotype as a random factor, using SPSS 22 (IBM SPSS Statistics). For analyzing height and diameter, the increment (end values – start values) was used, and start height and start diameter were set as covariates. When more than one leaf was measured per individual from the same treatment (e.g., leaf area), the

mean was used in the analyses. The normality of residuals was tested and data were \sqrt{x} - or $\ln(x)$ -transformed if necessary (Table S2 and S3). If data were not normally distributed, nonparametric tests were used.

A graphic vector analysis (GVA) was carried out to further elucidate the changes in leaf phenolic production and biomass accumulation in response to different treatments, according to published methods (Haase and Rose, 1995; Koricheva, 1999). Vector diagrams were built based on the relative values (treated mean/control mean) of the content (x), concentration (y) of each phenolic group and leaf biomass (z). The mean value in control treatment was used as the reference point ($x = y = z = 1$). The magnitude and direction of vectors indicate the magnitude and trend of the effects of the treatments. The directional changes show possible concentration, dilution, and excess or reduced synthesis. A concentration effect is the result of increasing relative concentration along with a decrease in relative content. On the other hand, a dilution effect is the result of decreasing relative concentration along with an increase in relative content. Excess synthesis increases both the relative content and concentration of a phenolic group, whereas reduced synthesis decreases both of them.

3. Results

3.1. Plant growth

Pyrene did not affect the height growth, diameter growth or leaf biomass of *P. tremula* seedlings, but it significantly decreased the stem biomass by 9% compared to controls under ambient conditions, and the decreases caused by pyrene were also present under elevated temperature (by 17%), elevated CO_2 (by 3%), and $T + \text{CO}_2$ (by 13%). (Figs. 1, 2, Table S2). Elevated temperature increased the height increment by 94% and 33%, diameter increment by 19% and 16%, leaf biomass by 43% and 35%, and stem biomass by 47% and 35%, for females and males, respectively when compared to ambient in both soil treatments (Figs. 1, 2). However, elevated CO_2 showed no effect on biomass, but decreased the height increment by 46% and 32%, and increased the diameter increment by 6% and 18% for females and males, respectively (Figs. 1, 2). Height growth and biomasses were higher under $T + \text{CO}_2$ than under any single treatment (significant $T \times \text{CO}_2$ interactions) (Figs. 1, 2, Table S2). Although no main effect of sex was detected, the increments in height and biomass were greater in females than in males under elevated temperature (significant $T \times \text{Sex}$ interactions) (Figs. 1, 2, Table S2). Pyrene did not change the effects of elevated temperature and CO_2 on height, diameter, or biomass, as there were no interactions between pyrene and $T/\text{CO}_2/T + \text{CO}_2$ (Table S2).

Pyrene reduced the leaf area (LA) by 6% compared to controls under ambient conditions, and the reductions were stronger under elevated temperature (38%), elevated CO_2 (37%), and $T + \text{CO}_2$ (42%) ($T \times \text{Pyrene}$, $\text{CO}_2 \times \text{Pyrene}$, $T \times \text{CO}_2 \times \text{Pyrene}$ interactions) (Table 1). In addition, the decrement of LA caused by pyrene was greater in males than in females (significant $\text{Pyrene} \times \text{Sex}$ interaction) (Table 1). Pyrene did not affect specific leaf area (SLA) under any climatic condition (Table 1). However, elevated temperature increased SLA in comparison with ambient, and the increment was greater in females than in males (significant $T \times \text{Sex}$ interaction) (Table 1). Elevated CO_2 decreased both LA and SLA, but elevated temperature eliminated the negative effects of elevated CO_2 (significant $T \times \text{CO}_2$ interactions) (Table 1).

3.2. Leaf phenolics

Three groups of phenolics were detected in the leaves of *P. tremula* seedlings, namely salicylates (74–86% of leaf phenolics, all treatments), flavonoids (8–19%), and phenolic acids (5–8%) (Fig. 3, Table S3). In uncontaminated soils, concentrations of total salicylates and total phenolics decreased under elevated temperature but increased under elevated CO_2 . Elevated CO_2 eliminated the negative effects of elevated

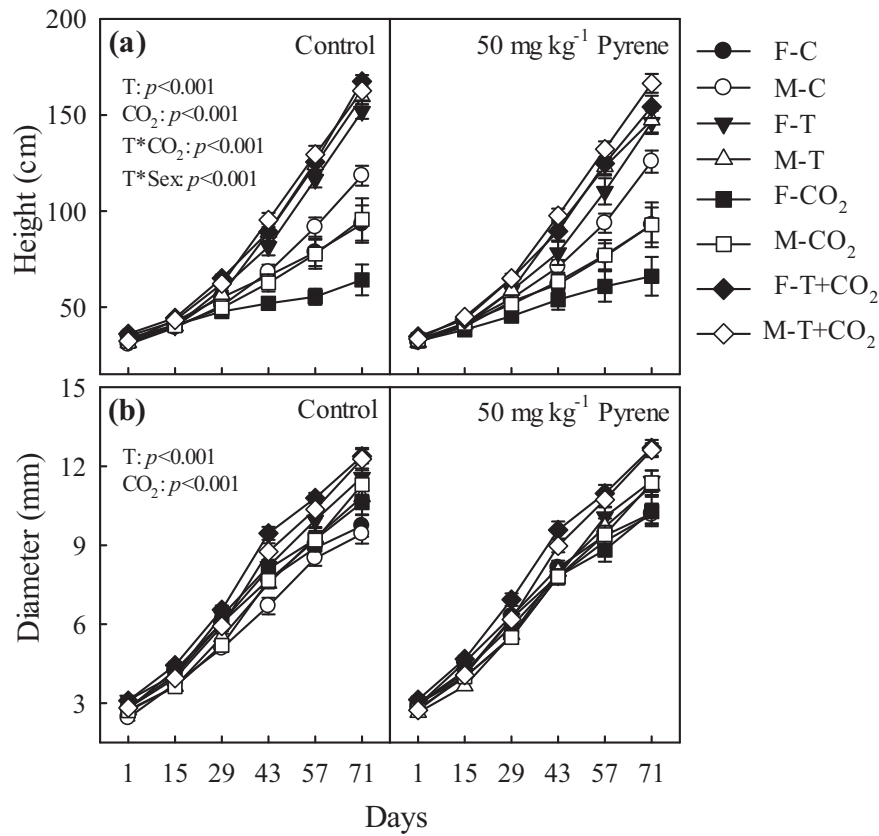


Fig. 1. Mean (\pm SE) height (a) and diameter (b) growth of females (F) and males (M) of *P. tremula* seedlings under control (C), elevated temperature (T), elevated CO₂ concentration (CO₂), and T + CO₂ conditions in different soil treatments. Statistically significant results ($p < 0.05$) from the linear mixed model are presented.

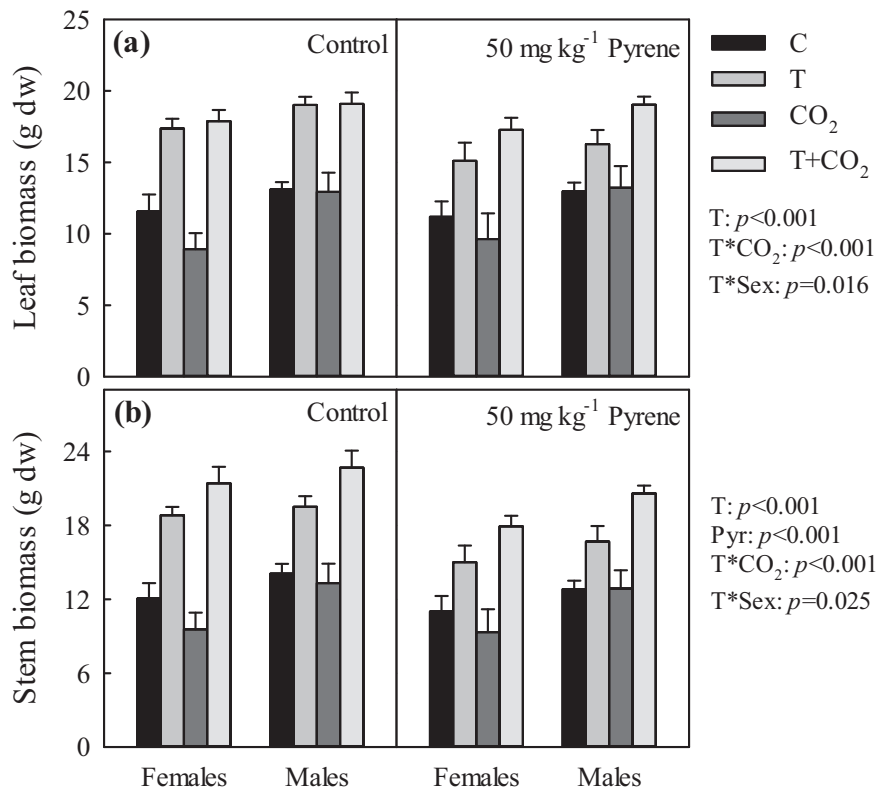


Fig. 2. Mean (\pm SE) leaf (a) and stem (b) biomass of *P. tremula* seedlings under control (C), elevated temperature (T), elevated CO₂ concentration (CO₂), and T + CO₂ conditions in different soil treatments. Statistically significant results ($p < 0.05$) from the linear mixed model are presented.

Table 1

Mean (\pm SE) leaf area (LA) and specific leaf area (SLA) of females and males of *P. tremula* seedlings under control (C), elevated temperature (T), elevated CO₂ concentration (CO₂), and T + CO₂ conditions in different soil treatments. *F*-values and levels of significance (*p*) from the linear mixed model for T, CO₂, Pyrene, Sex, and their interactions are presented. Statistically significant results are marked with bold (*p* < 0.05).

Pyrene (mg kg ⁻¹)		LA (cm ²)		SLA (cm ² g ⁻¹)	
		0	50	0	50
C	Females	130.2 \pm 8.2	134.0 \pm 11.3	409.5 \pm 5.9	407.5 \pm 1.7
	Males	161.9 \pm 0.7	150.1 \pm 4.4	495.7 \pm 13.1	459.5 \pm 16.6
T	Females	149.9 \pm 5.4	100.9 \pm 2.7	515.3 \pm 7.1	517.7 \pm 11.0
	Males	190.5 \pm 5.2	110.7 \pm 4.7	530.4 \pm 11.5	537.7 \pm 17.3
CO ₂	Females	138.9 \pm 9.3	88.9 \pm 4.7	336.6 \pm 8.3	337.3 \pm 7.9
	Males	147.5 \pm 7.4	92.8 \pm 3.5	375.2 \pm 11.2	340.4 \pm 21.5
T + CO ₂	Females	147.9 \pm 9.3	88.0 \pm 2.2	509.3 \pm 13.0	514.7 \pm 14.7
	Males	188.0 \pm 6.8	102.9 \pm 4.5	515.2 \pm 13.1	528.0 \pm 8.5
<i>F</i> _T (<i>p</i>)		1.368 (0.243)		305.027 (0.000)	
<i>F</i> _{CO₂} (<i>p</i>)		16.602 (0.000)		66.989 (0.000)	
<i>F</i> _{Pyrene} (<i>p</i>)		142.940 (0.000)		1.537 (0.216)	
<i>F</i> _{Sex} (<i>p</i>)		3.313 (0.119)		1.155 (0.324)	
<i>F</i> _{T×CO₂} (<i>p</i>)		5.730 (0.018)		49.072 (0.000)	
<i>F</i> _{T×Pyrene} (<i>p</i>)		21.832 (0.000)		3.222 (0.074)	
<i>F</i> _{T×Sex} (<i>p</i>)		1.537 (0.216)		4.519 (0.035)	
<i>F</i> _{CO₂×Pyrene} (<i>p</i>)		10.612 (0.001)		0.001 (0.976)	
<i>F</i> _{CO₂×Sex} (<i>p</i>)		1.275 (0.260)		2.602 (0.108)	
<i>F</i> _{Pyrene×Sex} (<i>p</i>)		5.195 (0.024)		2.268 (0.134)	
<i>F</i> _{T×CO₂×Pyrene} (<i>p</i>)		5.810 (0.017)		0.493 (0.483)	
<i>F</i> _{T×CO₂×Sex} (<i>p</i>)		1.816 (0.179)		2.203 (0.139)	
<i>F</i> _{T×Pyrene×Sex} (<i>p</i>)		1.375 (0.242)		3.565 (0.060)	
<i>F</i> _{CO₂×Pyrene×Sex} (<i>p</i>)		0.096 (0.757)		0.388 (0.534)	
<i>F</i> _{T×CO₂×Pyrene×Sex} (<i>p</i>)		0.011 (0.916)		0.305 (0.581)	

temperature (significant T × CO₂ interactions) (Fig. 3a, d, Table S3). Pyrene did not affect the concentrations of salicylates and total phenolics under ambient conditions, but it significantly increased them under elevated temperature (significant T × Pyrene interactions) (Fig. 3a, d, Table S3). Pyrene increased total phenolics by 16%, 1%, and

20% compared to controls under elevated temperature, elevated CO₂, and T + CO₂, respectively (Fig. 3d, Table S3). Elevated temperature decreased, while CO₂ increased the concentrations of total flavonoids and phenolic acids (Fig. 3b, c, Table S3). The concentration of total flavonoids was reduced by pyrene, and the reduction was greater under elevated temperature (significant T × Pyrene interaction). Elevated CO₂ also increased flavonoids more in females than in males in control soils, but not in pyrene-spiked soils (significant CO₂ × Pyrene × Sex interaction) (Fig. 3b, Table S3).

Pyrene decreased the concentrations of salicortin (57–67% of total salicylates), tremulacin (18–32% of total salicylates), quercetin 3-glucuronide (40–55% of total flavonoids), kaempferol 3-glucuronide (21–28% of total flavonoids), and neochlorogenic acid (50–65% of total phenolic acids) under ambient conditions. However, under elevated temperature pyrene increased salicortin, tremulacin, and neochlorogenic acid (significant T × Pyrene interactions) (Table S3). The reduction of quercetin 3-glucuronide caused by pyrene was stronger under elevated temperature (significant T × Pyrene interaction) (Table S3). Elevated temperature alone decreased quercetin 3-glucuronide and kaempferol 3-glucuronide, but increased tremulacin. Elevated CO₂ increased salicortin, tremulacin, quercetin 3-glucuronide, and neochlorogenic acid in both soil treatments (Table S3). Females had higher concentrations of kaempferol 3-glucuronide and neochlorogenic acid (Table S3).

GVA diagrams were made for the three phenolic groups to further study the changes in phenolic allocation under elevated temperature, elevated CO₂, and soil pyrene contamination (Fig. 4). In control soils, elevated CO₂ induced a concentration effect on leaf salicylates, flavonoids, and phenolic acids in females, but the synthesis of the three phenolic groups in males was increased by elevated CO₂ (Fig. 4a–f). Elevated temperature resulted in dilution effects on salicylates and phenolic acids, while T + CO₂ increased the accumulation of salicylates and flavonoids in both sexes in control soils (Fig. 4a–f). In both sexes, pyrene reduced the synthesis of salicylates and phenolic acids under ambient

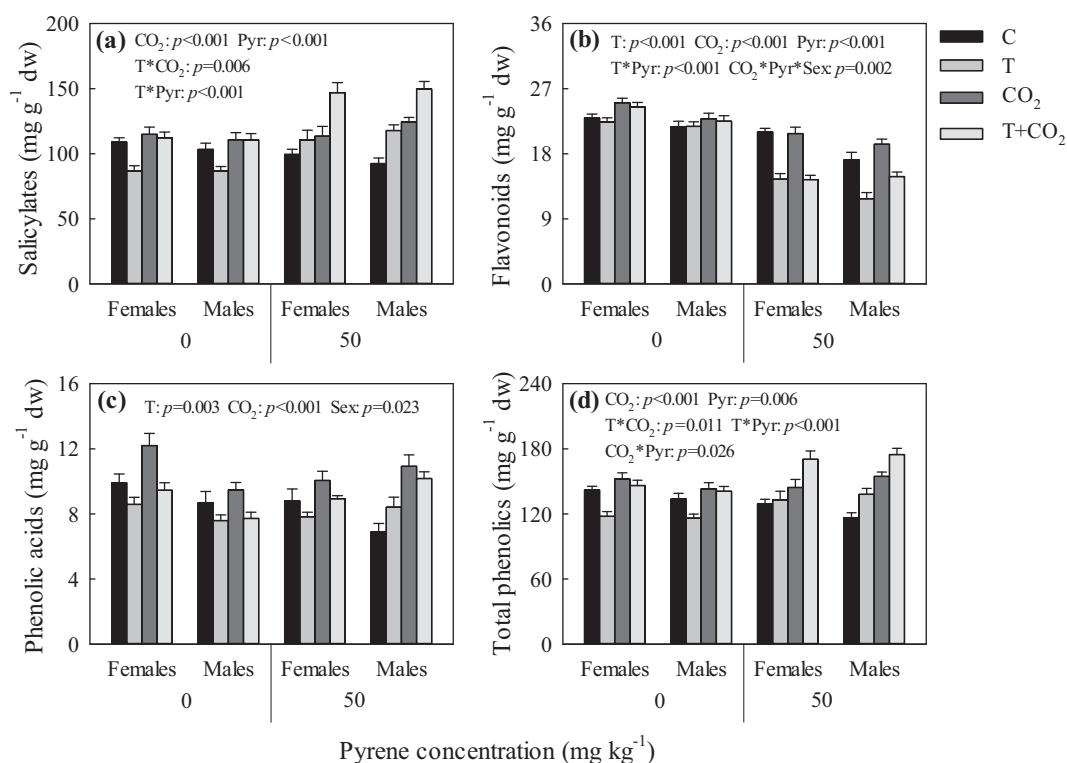


Fig. 3. Mean (\pm SE) concentrations of leaf total salicylates (a), flavonoids (b), phenolic acids (c), and total phenolics (d) in different sexes of *P. tremula* seedlings under control (C), elevated temperature (T), elevated CO₂ concentration (CO₂), and T + CO₂ conditions in different soil treatments. Statistically significant results (*p* < 0.05) from the linear mixed model are presented.

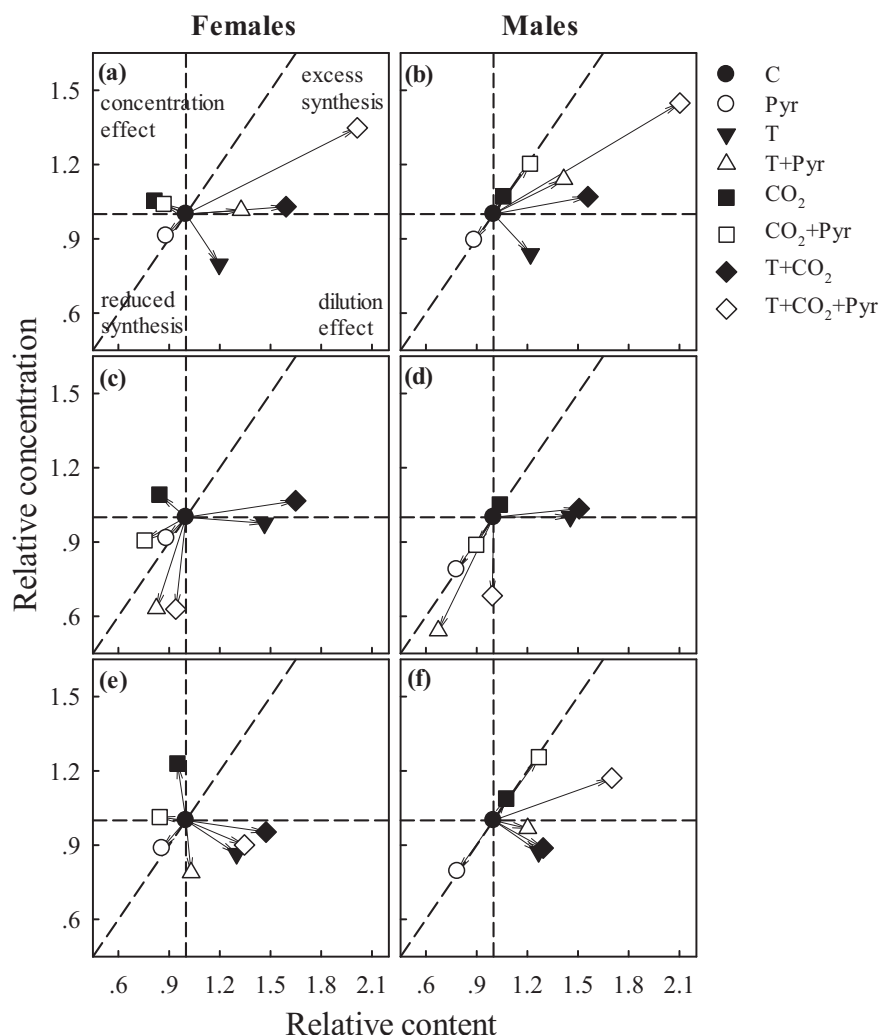


Fig. 4. Graphic vector diagrams of total salicylates (a, b), flavonoids (c, d), and phenolic acids (e, f) in leaves of females and males of *P. tremula* seedlings under control (C), elevated temperature (T), elevated CO₂ concentration (CO₂), and T + CO₂ conditions without and with pyrene (Pyr) in soil. The direction of the vector arrows indicates the trend of the treatments' effects (concentration, dilution, and excess or reduced synthesis); the length of the vector arrows indicates the magnitude of the effects. The oblique dashed lines represent reference lines for no change in leaf biomass; the vertical dashed lines represent the reference lines for no change in phenolic content; and the horizontal dashed lines represent the reference lines for no change in phenolic concentration.

conditions, while it reduced the synthesis of flavonoids in all climate treatments (Fig. 4a–f). However, the synthesis of salicylates in both sexes and that of phenolic acids in males were increased by pyrene under T + CO₂ (Fig. 4a, b, f).

3.3. Residual pyrene in soil

The concentration of pyrene in pyrene-spiked soils was significantly ($p < 0.05$) decreased after 11 weeks of planting (Fig. 5). The residual pyrene in soils was higher (28% and 45% for females and males, respectively) under elevated CO₂ than under ambient conditions (Fig. 5). There was, however, no significant difference in residual pyrene concentration between control and elevated temperature/combined T + CO₂ treatments (Fig. 5). There were no significant effects of plant sex on soil pyrene concentration, nor any interaction between pyrene and climate factors (Fig. 5).

4. Discussion

PAHs impact plant growth through both air-leaf pathways and soil-root pathways. The PAH stress responses in plants include disturbances in photosynthesis, carbon allocation, antioxidant enzyme activities,

basic metabolism, protein synthesis, and signal transduction (Alkio et al., 2005; Desalme et al., 2013; Zhang et al., 2013). In this study,

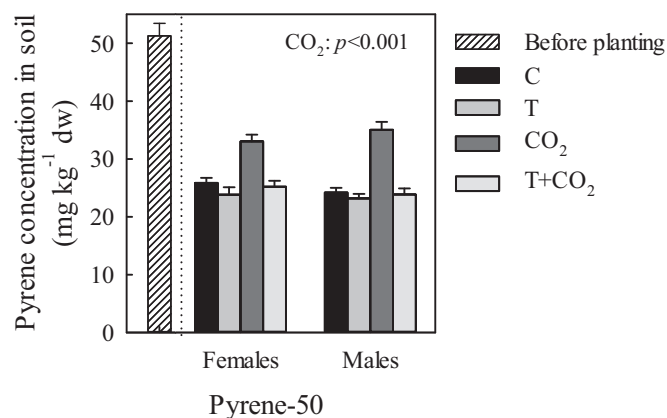


Fig. 5. Mean (\pm SE) pyrene concentrations in pyrene-spiked soils before and after planting different sexes of *P. tremula* seedlings under control (C), elevated temperature (T), elevated CO₂ concentration (CO₂), and T + CO₂ conditions. Statistically significant results ($p < 0.05$) from the linear mixed model are presented.

pyrene-spiked soil moderately decreased shoot biomass and leaf area, but did not affect other growth parameters of *P. tremula* seedlings. Our previous study using a similar pyrene-spiked (50 mg kg^{-1}) soil showed that pyrene decreased height growth and shoot biomass of *Picea abies* seedlings (Zhang et al., 2018).

Elevated temperature increased plant growth parameters including height, diameter, biomass, and specific leaf area, in accordance with previous studies of *P. tremula* (Randriamanana et al., 2015; Sobuj et al., 2018). However, elevated CO_2 reduced height and specific leaf area, but increased diameter in this study. Similar results from studies on *P. abies* (Sallas et al., 2003), *Larix kaempferi* (Yazaki et al., 2004), and *P. tremula* (Sobuj et al., 2018) have also been reported. Growth of trees at high latitude may be temperature-limited and will benefit from moderate elevation of temperature (Way and Oren, 2010). The strong responses of plant growth to elevated temperature in our study suggest that the optimum growth temperature for *P. tremula* has not yet been reached, as the mean ambient temperature was 15.2°C during the experiment. Elevated CO_2 may result in a short apical portion of the main stem of *P. tremula* (Sobuj et al., 2018), which is probably because of the effects of elevated CO_2 on hormonal production or transportation in the shoot apex. Increased biomass and specific leaf area under elevated temperature, as well as decreased specific leaf area under elevated CO_2 , may be associated with greater transpiration stress, reflecting the demand for increased water use efficiency (Sallas et al., 2003).

In our study, elevated temperature decreased, while elevated CO_2 increased the concentration of leaf total phenolics in *P. tremula* seedlings in uncontaminated soils. Pyrene did not affect leaf total phenolics under ambient conditions, but it increased those under elevated temperature and $\text{T} + \text{CO}_2$. Plant growth and secondary metabolism are greatly affected by environmental stresses which can divert substrates from primary metabolism into secondary defensive metabolites (Lattanzio et al., 2012). Thus, there may be trade-offs between growth and defense because plants have limited resources to support the physiological processes (Herms and Mattson, 1992; Lattanzio et al., 2012). Similar trade-offs caused by elevated temperature and CO_2 were also found in previous studies on *Salix myrsinifolia*, *Betula pendula*, and *P. tremula* (Veteli et al., 2007; Randriamanana et al., 2015). PAH (e.g., phenanthrene) has also been reported to cause a carbon metabolic shift from plant primary metabolism to secondary metabolism (Desalme et al., 2013). In our previous study on *P. abies*, the concentrations of total phenolics in needles and stems were significantly decreased by pyrene, and the decrease was greater under elevated temperature (Zhang et al., 2018). This indicates that different tree species respond differently to pyrene in combination with climate factors.

Salicylates are the main phenolic compounds in the leaves of *P. tremula* seedlings and they play an important role in protecting plants against generalist herbivores (Randriamanana et al., 2014; Sobuj et al., 2018). In this study, salicylates constituted 74–86% of leaf total phenolics in all treatments. Among the salicylates, salicortin and tremulacin are the most abundant in *Populus* species. They are also the most biologically active compounds because of their cyclohexenone carboxylic acid functional group (e.g. Lindroth and St. Clair, 2013). Although leaves of *P. tremula* prioritized the accumulation of salicylates, we also detected synthesis of flavonoids and phenolic acids. Flavonoids and phenolic acids have been considered good antioxidants that can scavenge or suppress the formation of reactive oxygen and/or nitrogen species (Dai and Mumper, 2010). The various effects of pyrene, elevated temperature and CO_2 , as well as their interactions on the three groups of phenolics, suggest that plants can adapt to future environmental stresses by regulating the growth and production of chemical defense.

For dioecious plant species, sexes prioritize the allocation of resources differently, and the sexual variations can be affected by abiotic stresses (Nybakken et al., 2012; Maja et al., 2016). In our study, no significant sex differences were detected, but males tended to be taller and had bigger biomass than females under ambient conditions

(Figs. 1, 2). However, the growth of females benefited more from elevated temperature. This finding is in agreement with the results of Randriamanana et al. (2015), and may indicate that the growth of females was more limited by temperature. Pyrene had no sex-specific effects on the growth of *P. tremula* seedlings. Previous studies on dioecious plants have noted that females allocate more resources to defensive phenolics than males (Nybakken and Julkunen-Tiitto, 2013; Randriamanana et al., 2015). However, we found no significant sexual differences in leaf total phenolics in this study, but concentrations of many individual phenolic compounds were higher in females than in males. Although pyrene had limited sex-specific effects on the concentration of leaf total phenolics, interactions between sex and pyrene/ CO_2 were detected for several individual phenolics. The small sexual differences indicate that both sexes of *P. tremula* have better chemical defense capability to adjust to the tested pyrene contamination under elevated temperature and CO_2 .

Plants can promote microbial biodegradation of pyrene in soil by plant-secreted enzymes in the root zone (Liste and Alexander, 2000; Lu et al., 2014). In addition, soil microorganisms play a vital role in the decomposition of organic matter (Drissner et al., 2007). In this study, the concentration of residual pyrene in soils was higher under elevated CO_2 than under ambient, elevated temperature, and combined $\text{T} + \text{CO}_2$. Studies on the effect of elevated atmospheric CO_2 on the degradation of PAHs in soils are scarce. Elevated atmospheric CO_2 may not directly influence soil microorganisms as the concentration of CO_2 in soil is much higher than in the atmosphere, but they can indirectly affect root growth and rhizodeposition which are closely associated with the soil microbial community (Kandeler et al., 1998). In addition, secondary plant metabolites, such as salicylates, have an important role in developing PAHs-degrading bacteria (Singer et al., 2003). However, not all PAH degraders can use salicylates as growth substrates, because of the considerable heterogeneity among bacteria (Bogan et al., 2001). Our previous study noted that elevated CO_2 significantly increased total PAH residuals (i.e., decelerated the degradation of PAHs). This could be explained by alterations of soil microbial community composition and decreases in the biomass of Gram-positive bacteria, which was the most important group of soil microbes in degrading PAHs (Ai et al., unpublished data). However, further studies are needed to investigate microbial communities in higher taxonomic resolution to identify the underlying mechanisms. Temperature changes can directly affect soil microbial diversity, growth, mineralization rate, and enzyme activities (Tschirko et al., 2001). Iqbal et al. (2007) demonstrated that elevated temperature (42°C vs. 21°C) accelerated the bioremediation of PAHs in soils, which might be because elevated temperature increased the ability of indigenous microorganisms to degrade PAHs and decreased the adsorption so that more organic materials were available for microorganisms to degrade. However, the temperature enhancement (1.8°C on average: 17.0°C vs. 15.2°C) in our study was too small to change the degradation of pyrene in soil.

5. Conclusions

Pyrene of a certain concentration decreased growth of *P. tremula* seedlings, and the decrease in leaf area was more severe under elevated temperature, elevated CO_2 , and combined $\text{T} + \text{CO}_2$. Pyrene did not affect the concentration of leaf total phenolics under ambient conditions, but significantly increased it under elevated temperature and $\text{T} + \text{CO}_2$. In addition, pyrene had little sex-specific effects on the growth and leaf phenolics of *P. tremula* seedlings. The concentration of residual pyrene in pyrene-spiked soils was higher under elevated CO_2 than under ambient, elevated temperature, and $\text{T} + \text{CO}_2$. We assume that both sexes of *P. tremula* can build their protection mechanisms by regulating growth and metabolism to adjust to the stress of the tested pyrene contamination under elevated temperature and CO_2 , but the risk of pyrene to plants still exists in the future.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.scitotenv.2018.03.344>.

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